11-13-00

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JC960 U.S. PTO 11/10/00

Practitioner's Docket No. 701826/50990 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

JC608 U.S. PTO 09/709249 11/10/00

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s): Andrew R. CROW, John FREEDMAN, Barbara HANNACH, Allen H. LAZRUS

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): A METHOD FOR PREVENTING AND INHIBITING HUMAN HLA ALLOIMMUNE RESPONSE TO PLATELET TRANSFUSION

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date November 10, 2000, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL565099952US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Patricia W. Turner
(type or print name of person mailing paper)
Patricia W. Turner
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

****WARNING:*** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

Type of Application

This new application is for a(n)

(check one applicable item below)

[X] Original (nonprovisional)
[ ] Design
[ ] Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

[ ] Divisional.
[ ] Continuation.
[ ] Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

[ ] The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

__19__ Pages of Specification
__2__ Pages of Claims
__4__ Sheets of Drawing

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988. (1990 O.G. 57-62).*

NOTE: *"Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page. . ." 37 C.F.R. § 1.84(c)).*

(complete the following, if applicable)

[ ] The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

[X] Formal
[ ] Informal

B. Other Papers Enclosed

____ Pages of declaration and power of attorney
__1__ Pages of Abstract

____ Other

4. Additional Papers Enclosed

[ ] Amendment to claims

[ ] Cancel in this applications claims _____before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[ ] Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)

[ ] Preliminary Amendment
[ ] Information Disclosure Statement (37 C.F.R. § 1.98)
[ ] Form PTO-1449 (PTO/SB/08A and 08B)
[ ] Citations
[ ] Declaration of Biological Deposit
[ ] Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
[ ] Authorization of Attorney(s) to Accept and Follow Instructions from Representative
[ ] Special Comments
[ ] Other

5. Declaration or Oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. § 1.63(d)(1)-(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).

[ ] Enclosed

Executed by

(check **all** *applicable boxes)*

[ ] inventor(s).
[ ] legal representative of inventor(s). 37 C.F.R. § 1.42 or 1.43.
[ ] joint inventor or person showing a proprietary interest on behalf of inventor who

refused to sign or cannot be reached.

[ ] This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

[X] Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

[ ] Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e), can be filed subsequently).

[ ] Showing that the filing is authorized.
(not required unless called into question. 37 C.F.R. § 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

[ ] The same.

or

[ ] Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
[ ] is submitted.
[ ] will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).

[X] English
[ ] Non-English

[ ] The attached translation includes a statement that the translation is accurate. 37

C.F.R. § 1.52(d).

8. Assignment

[ ] An assignment of the invention to _____

_____

[ ] is attached. A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

[ ] will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "STATEMENT UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. no.	Filed
Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

[ ] is (are) attached.

[ ] will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. § 1.16)

A. [X] Regular application

CLAIMS AS FILED					
Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. § 1.16(a) $760.00
Total Claims (37 C.F.R. § 1.16(c))		- 20 =	x	$ 18.00	
Independent Claims (37 C.F.R. § 1.16(b))		- 3 =	x	$ 78.00	
Multiple Dependent Claim(s), if any (37 C.F.R. § 1.16(d))			+	$260.00	

[ ] Amendment cancelling extra claims is enclosed.
[ ] Amendment deleting multiple-dependencies is enclosed.
[ ] Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d).

Filing Fee Calculation $_____

B. [ ] Design application
($310.00—37 C.F.R. § 1.16(f))
Filing Fee Calculation $_____

C. [ ] Plant application
($480.00—37 C.F.R. § 1.16(g))
Filing Fee Calculation $_____

11. Small Entity Statement(s)

[ ] Statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27 is (are)

attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

(complete the following, if applicable)

[ ] Status as a small entity was claimed in prior application _____/_____, filed on _____ from which benefit is being claimed for this application under:

35 U.S.C. § [ ] 119(e),
[ ] 120,
[ ] 121,
[ ] 365(c),

and which status as a small entity is still proper and desired.

[ ] A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $_____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C.F.R. § 1.28(a).

12. Request for International-Type Search (37 C.F.R. § 1.104(d))

(complete, if applicable)

[ ] Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

[X] Not Enclosed

[ ] No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

[ ] Enclosed

[ ]	Filing fee	$_____
[ ]	Recording assignment ($40.00; 37 C.F.R. § 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.")	$_____
[ ]	Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 C.F.R. §§ 1.47 and 1.17(i))	$_____
[ ]	For processing an application with a specification in a non-English language ($130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k))	$_____
[ ]	Processing and retention fee ($130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l))	$_____
[ ]	Fee for international-type search report ($40.00; 37 C.F.R. § 1.21(e))	$_____

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. § 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total Fees Enclosed $_____

14. Method of Payment of Fees

[ ] Check in the amount of $_____.

[ ] Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should <u>not</u> be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim*

charges are authorized.

[ ] The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No._____.

[ ] 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)

[ ] 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

[ ] 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

[ ] 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).

[ ] 37 C.F.R. § 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

[ ] 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b)).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . issue fee." From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[ ] Credit Account No. _____.

[ ] Refund

SIGNATURE OF PRACTITIONER

Reg. No. 34,235

David S. Resnick
(type or print name of practitioner)

Tel. No.: 617.345.6057

Nixon Peabody LLP
101 Federal Street
P.O. Address

Customer No.:

Boston, MA 02110

[X] **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

[X] Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added _____

[ ] Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

[ ] Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

[ ] Plus "Assignment Cover Letter Accompanying New Application"
Number of pages added _____

[ ] **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

[X] This transmittal ends with this page.

Practitioner's Docket No. 701826/50990 *PATENT*

ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 C.F.R. § 1.78.

17. Relate Back

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

(complete the following, if applicable)

[ ] Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).

[X] "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
60 / 164,777	November 12, 1999 "
/	"
/	"

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

[ ] "This application is a

[ ] continuation

[ ] continuation-in-part

[ ] divisional

of copending application(s)

[ ] application number 0 /_____ filed on _____"

[ ] International Application _____ filed on _____ and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

[ ] "The nonprovisional application designated above, namely application _____/_____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	**FILING DATE**
_____/_____	_____"
_____/_____	_____"
_____/_____	_____"

[ ] Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed

The certified copy(ies) has (have)

[ ] been filed on _____, in prior application 0 /_____, which was filed on _____.

[ ] is (are) attached.

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).*

19. Maintenance of Copendency of Prior Application

NOTE: *The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).*

A. [ ] Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application,** if the period set in the prior application has run.)*

[ ] A petition, fee and response extends the term in the pending **prior** application until _____

[ ] A **copy** of the petition filed in prior application is attached.

B. [ ] Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

[ ] A conditional petition for extension of time is being filed in the pending **prior** application.

[ ] **A copy** of the conditional petition filed in the prior application is attached.

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) [ ] This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

[ ] the same.

[ ] less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

_____

(type name(s) of inventor(s) to be deleted)

(b) [ ] This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

[ ] the same.

[ ] the following additional inventor(s) have been added:

_____

(type name(s) of inventor(s) to be deleted)

(c) [ ] The inventorship for all the claims in this application are

[ ] the same.

[ ] not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

[ ] is submitted.

[ ] will be submitted.

21. Abandonment of Prior Application *(if applicable)*

[ ] Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in- part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b), 6th ed., rev.2.*

NOTE: *Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.*

(check the next item, if applicable)

[ ] There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

[ ] Applicant has established small entity status by the filing of a statement in parent application /_____ on _____.

[ ] A copy of the statement previously filed is included.

WARNING: See 37 CFR § 1.28(a).

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

[ ] A notification of the filing of this
(check one of the following)

[ ] continuation

[ ] continuation-in-part

[ ] divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

A METHOD FOR PREVENTING AND INHIBITING HUMAN HLA ALLOIMMUNE RESPONSE TO PLATELET TRANSFUSION

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a method for preventing and /or inhibiting HLA alloimmune response to platelet transfusion, by presensitizing platelets with at least one monoclonal HLA antibody.

(b) Description of Prior Art

Many patients who receive platelet transfusions become alloimmunized, rendering them refractory to subsequent platelet transfusions. It is thought that "contaminating" HLA Class II bearing antigen presenting cells (APC) augment the production of these alloantibodies and various methods have been used to inactivate or remove these "contaminating" cells, including the use of ultraviolet radiation and leucofiltration. Although these methods have been successful at reducing the incidence of primary alloimmunization, many multi-transfused patients still become alloimmunized. For those patients already alloimmunized by prior transfusion or pregnancy, even leucodepleted platelets can stimulate a secondary alloimmune response. Animal studies suggest that extreme leucodepletion may be detrimental to inhibiting immune responses to transfusions, suggesting that the ability of leucodepletion to decrease alloimmunization may have reached its threshold.

Antigen-specific IgG, when injected at the time of antigen exposure, can induce a strong suppression of the immune response. The immunosuppressive effect is particularly effective with large antigen systems, such as red blood cells, and this is currently applied to the prevention of fetal erythroblastosis in Rh

negative women by administration of anti-D IgG. Pretreatment of whole blood with polyclonal alloantisera has been shown to prevent alloantibody production in rat models of transfusion. More refined studies in rats have shown that pretreatment of platelets or leukocytes with alloantisera also inhibits the alloimmune response to platelet transfusion.

Antibody/antigen complexes can inhibit immune responses and it has been hypothesized in the immunological literature that this down-regulation of humoral responses is likely contributed to by a negative feedback pathway mediated by B cell Fcγ receptor (FcγR) co-crosslinking with the B cell Ig receptor (BCR), resulting in the B cell entering a "non-responsive" state mediated by activation of a negative feedback pathway at the level of BCR signaling.

An alternate theory developed is that alloimmune serum from alloimmunized individuals contains elevated levels of an anti-IgG (i.e. an IgG rheumatoid factor (RF)) and this IgG RF contributes to or mediates a decrease in the alloimmune response. Purified IgG RF from the serum of alloimmunized rats exerts immunosuppressive effects *in vivo* and *in vitro*.

The inventors have shown previously that SCID mice, engrafted with human (Hu) peripheral blood lymphocytes (PBL) from alloimmunized donors are a valuable tool for studying alloimmunization and that transfusion of these Hu-PBL-SCID mice with human alloimmune sera presensitized platelets results in a decreased alloantibody response to further untreated platelet transfusions (Crow AR, et al., *Br J Haematol* **104**:919, 1999).

In the present invention, a single dose of platelets presensitized with monoclonal HLA Class I antibody (either depleting or non-depleting) abrogated the alloantibody response to five subsequent untreated platelet challenges. FcR mediated B cell down regulation was not required for the alloimmune inhibition observed, since F(ab')2 fragments of monoclonal anti-HLA-A,B,C antibody completely abrogated the immune response whereas platelets treated with platelet-specific antibody or control murine IgG had no inhibitory effect.

It would be highly desirable to be provided with a new approach for inhibiting the human alloimmune response to platelet transfusion.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a new approach for inhibiting the human alloimmune response to platelet transfusion.

In accordance with the present invention there is provided a new method for inhibiting the human alloimmune response to platelet transfusion.

Since monoclonal antibodies can be made by recombinant means and the fine specificity of the antibody is not critical to inhibit alloimmunization, the present invention provides a new and practical approach for inhibiting the human alloimmune response to platelet transfusion.

In accordance with the present invention there is provided a method for preventing HLA alloimmune response to platelet transfusion, comprising the step of presensitizing platelets with at least one monoclonal antibody against HLA, a portion thereof, or β2-microglobulin, wherein the platelets if administered

to a patient prevent an HLA alloimmune response from the patient.

The monoclonal antibody can be for example W6/32, L368, and MA2.1.

In accordance with the present invention there is also provided a method for inhibiting an HLA alloimmune response to platelet transfusion. The method comprises the steps of:

a) presensitizing platelets with at least one monoclonal antibody against HLA or a portion thereof; and

b) transfusing with the presensitized platelets of step a) to a patient, the presensitized platelets inhibiting an HLA alloimmune response from the patient.

The HLA alloimmune response can still be prevented after at least two transfusions from the patient.

The term "platelets" in the instant application is intended to also include, without limitation, platelet concentrates, platelet substitutes, platelet rich plasma, platelet poor plasma, lyophilized platelets, platelets fragments, red blood cells, red blood cell concentrates, leukocytes and buffy coats.

The monoclonal antibodies useful in the method of the present invention include, without limitation, monoclonal antibodies against either Public or Private epitopes of HLA. The monoclonal antibodies do not have necessarily to be against HLA as monoclonal antibodies against the B-2 microglobulin portion of HLA are also effective at alloimmune inhibition.

The expression "monoclonal antibodies" also meant to include without limitation murine monoclonal antibodies, recombinant MAbs, humanized MAbs, single chain MAbs, bispecific MAbs where one epitope is HLA

or B-2M, $F(ab)'_2$ and F(ab) fragments of these monoclonal antibodies.

The present invention can be used to protect or prevent alloimmunization. However, the method of the present invention can also be used for preventing refractoriness to subsequent transfusions in alloimmunized patients.

In accordance with the present invention, there is provided a method for preventing refractoriness to subsequent transfusions in an alloimmunized patient, comprising a) presensitizing platelets with at least one monoclonal antibody against HLA or a portion thereof, and b) transfusing the alloimmunized patient with the presensitized platelets of step a), the presensitized platelets preventing refractoriness to the transfusion.

In accordance with the present invention, there is provided a method for preventing an alloimmune disease or an alloresponse. The method comprises the steps of a) presensitizing platelets with at least one monoclonal antibody against HLA or a portion thereof; and b) transfusing with the presensitized platelets of step a) to a patient. The presensitized platelets inhibit an HLA alloimmune response from the patient.

The alloresponse may be for example an organ transplantation-related complication, such as an organ rejection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A to 1F are graphs illustrating saturation of transfused platelets by monoclonal antibodies;

Fig. 1G is a graph illustrating saturation of alloantibody-containing Hu-PBL-SCID sera;

Fig. 2 is a scattergram plot illustrating inhibition of the alloantibody response by monoclonal HLA Class I antibody treated platelets;

Fig. 3 illustrates the effect of monoclonal antibodies on platelet immunogenicity; and

Fig. 4 illustrates the effect of F(ab')2 fragment of monoclonal antibody W6/32 on platelet immunogenicity.

DETAILED DESCRIPTION OF THE INVENTION

Previous results have shown that presensitization of donor platelets, white blood cells or whole blood with allo-specific IgG results in a diminished immune response against subsequent transfusions of platelets. To better understand the mechanism of how alloantibody presensitization results in a decreased alloimmune response, and since monoclonal antibodies do not contain contaminants as do polyclonal antibody preparations, the allospecific inhibition in the absence of the effect of the inhibitory IgG(s) can thus be examined in the present application. Murine monoclonal antibodies directed to polymorphic and non-polymorphic regions of human HLA as well as platelet-specific molecules were used in the present invention. Accordingly, it is demonstrated in the present application that presensitization with anti-human HLA Class I antibodies as well as $_2$M-specific antibody could protect against alloantibody production to 5 subsequent untreated platelet challenges. Use of depleting (complement fixing), non-depleting, high or low FcR binding antibodies or F(ab')2 fragments of HLA-specific antibody also resulted in complete inhibition of alloantibody. This protection was not seen when the platelets were presensitized with monoclonal antibodies to CD42a

(GPIX), CD32 (low affinity IgG-Fcγ receptor) or murine IgG; thus, this inhibition was therefore antigen specific and independent of complement-fixation or antibody-mediated Fc receptor dependent immunoregulatory effects. This inhibition was not dependent on HLA fine specificity, since antibodies directed at the β_2M portion of HLA class I were as effective as antibodies against any of the HLA-regions (either polymorphic or non-polymorphic regions) of class I. In accordance with the present invention, a single regime of HLA Class I specific monoclonal antibody presensitized platelets completely inhibits alloimmunization to further transfusions and offers an approach to preventing alloimmunization.

Monoclonal HLA-A2 antibody-treated platelets inhibit alloantibody production

Previous work showed that human polyclonal alloantisera to HLA-A2 could decrease production of alloantibody to HLA-A2 antigen (Crow AR, et al., *Br J Haematol* **104**:919, 1999). To determine if a monoclonal antibody could achieve the same effect, the inventors employed a murine monoclonal antibody (MA2.1), specific for HLA-A2 (see Table 1).

Table 1
Characteristics of Sensitizing Antibodies

Antibody	Subclass	Specificity	Complement fixing	FcγRII Binding[1]	Epitope
W6/32	IgG_{2a}	HLA-A,B,C	+	+	2/ 3
MA2.1	IgG_1	HLA-A2	-	++++	1
L368	IgG_1,k	$_2$ M	-	++++	$_2$M
IV.3	IgG_{2b}	CD32 (FcγRII)	+	+++	----
AN51	IgG_{2a},k	CD42a (GPIX)	+	+	----

[1] FcγRII binding of murine IgG, highest to lowest affinity: IgG1, 2b>>2a, 3

Hu-PBL-SCID mice were successfully engrafted as determined by the presence of human IgG in serum. Mice engrafted with human lymphocytes from an HLA-A2 alloimmunized donor and challenged with HLA-A2 positive platelets produced alloantibody detectable at day 7 post engraftment and increased over time to day 24 (Fig. 2, O) as compared with unchallenged mice (Fig. 2, Δ). However, when the first platelet challenge was presensitized with a saturating dose of a murine monoclonal antibody to HLA-A2 (Fig. 1A), there was no alloantibody response to 5 subsequent untreated platelet challenges (Fig. 2, ; $p<0.001$ at all days except day 10, $p=0.02$ and day 18, $p=0.002$). Presensitized platelets did not decrease overall IgG production in the mice (1.8 ± 0.7 mg/ml) compared with untreated platelets (2.1 ± 0.7 mg/ml).

In Figs. 1A to 1G, platelets were incubated with serial dilutions of antibody (Fig. 1A: anti-HLA-A2, Fig. 1B: anti-HLA, Fig. 1C: anti-β_2M, Fig. 1D: anti-FcR, Fig. 1E: anti-CD42a, Fig. 1F: F(ab')2 anti-HLA). The x-axis shows antibody dilution, the y-axis

represents antibody binding as assessed by flow cytometry. The arrow indicates the amount of antibody used to presensitize the platelets prior to transfusion. Fig. 1G represents the dilution of alloantibody-containing Hu-PBL-SCID sera used for alloantibody detection and blocking experiments.

In Fig. 2, SCID mice were engrafted with lymphocytes from the first donor, making HLA-A2 specific antibodies. Engrafted Hu-PBL-SCID mice were either not further manipulated (Δ), challenged twice weekly (arrow) with HLA-A2 positive platelets (O), or challenged with monoclonal HLA-A2 antibody treated platelets, followed by 5 subsequent untreated platelet challenges (□) as above. The x-axis represents days post engraftment; y-axis is alloantibody binding to HLA-A2 positive PBLs in arbitrary mean fluorescence units. Cumulative data from 2 separate experiments are illustrated, n=10 for all groups.

Monoclonal antibodies to HLA class I, but not to platelet-specific antigens, inhibit the alloimmune response

Hu-PBL-SCID mice engrafted with lymphocytes from either of the two alloimmunized donors were challenged with platelet preparations presensitized with saturating doses of antibodies to HLA Class I (Figs. 1A to 1C) and other platelet surface antigens (Figs. 1D and 1E), followed by repeated untreated platelet challenges. Sera from the engrafted mice were analyzed at 21 days post engraftment for the presence of alloantibody. These Hu-PBL-SCID mice, when challenged with standard platelet preparations produced alloantibody as assessed by flow cytometry (Fig. 3).

In Fig. 3, SCID mice were engrafted with lymphocytes from the HLA-A2 or polyspecific alloimmunized donor and challenged as in Figs. 1A to

1G with HLA-A2 positive platelets or pooled platelets expressing multiple HLA alleles. Platelets were either untreated or pretreated with the monoclonal antibodies listed on the x-axis for the first challenge only. The y-axis represents alloantibody binding to target PBLs at 21 days post engraftment. The horizontal bar represents the mean fluorescence for the specified treatment groups.

Pretreatment of platelet preparations with murine IgG, CD42a-specific antibody, or FcγRII specific antibody, did not significantly decrease alloantibody production to further untreated platelet preparations compared to the positive control, untreated platelets ($p=0.92$ for mIgG, $p=0.21$ for CD42a antibody, $p=0.40$ for FcγR antibody). In contrast, platelets presensitized with either a monoclonal antibody to a polymorphic HLA epitope present on all HLA Class I molecules (HLA-A,B,C), a non-polymorphic epitope (HLA-A2), or the $_2$M invariant chain (Table 1), induced no alloantibody production to further untreated platelet challenges (Fig 3; $p<0.0001$ for HLA-A,B,C and $_2$M antibodies; $p<0.002$ for HLA-A2 antibody). The total serum human IgG levels were not different in mice transfused with antibody-treated platelets compared to those receiving untreated platelets.

Alloantibody inhibition by monoclonal antibodies is not FcR dependent

To determine if the alloantibody inhibition was associated with Fcγ R dependent effects, the first platelet challenge was either untreated or presensitized with saturating doses of whole anti-HLA antibody or a highly purified F(ab')2 fragment of the polymorphic HLA-A,B,C binding antibody (Figs. 1B and 1F). In contrast to the untreated platelet challenge group, platelets presensitized with either whole

antibody or F(ab')2 fragment failed to induce an alloantibody response to further untreated platelet challenges at 21 days post engraftment (Fig. 4; $p<0.0001$ for whole antibody; $p=0.003$ for F(ab')2 fragment). Anti-HLA antibodies were also able to inhibit alloantibody production regardless of their ability to bind host FcR (Table 1); W6/32 was as effective at alloimmune inhibition as MA2.1 or L368 All groups produced equivalent levels of overall IgG.

In Fig. 4, mice were engrafted as in Fig. 2, and challenged with untreated platelets or platelets treated with whole or F(ab')2 fragment of W6/32 for the first challenge only.

Monoclonal HLA class I antibodies do not sterically hinder binding of human alloantibodies

The HLA-A,B,C and β_2M monoclonal antibodies do not react with the 1 hypervariable region of HLA and would therefore not be expected to sterically hinder an immune response against HLA allo-regions. Nevertheless, these antibodies were tested as to whether or not they interfere with the binding to HLA by the allospecific sera from Hu-PBL-SCID mice and vice versa. Sera from platelet-challenged Hu-PBL-SCID mice (Table 2) and the monoclonal HLA antibodies used in these experiments (Table 3) were titrated with HLA-A2 positive PBLs and analyzed by flow cytometry to determine the minimum dose needed to saturate target cells.

Table 2

Inability of monoclonal HLA antibodies to block binding of human HLA antibodies

	Pre-incubation		
W6/32	L368	MA2.1	
Nil	1099 ± 561	1240 ± 705	473 ± 254
allosera	1332 ± 510	1336 ± 509	536 ± 209

Target cells were pre-incubated with nothing (Nil) or with monoclonal antibodies followed by incubation with Hu-PBL-SCID sera at concentrations specified in Fig. 1G and as described herein. Numbers represent mean channel fluorescence intensity ± S.E.M. from 3 experiments.

Table 3
Inability of human alloantibodies to block monoclonal HLA antibody binding

Pre-incubation with SCID sera	
Nil	362 ± 132
W6/32	384 ± 167
L368	557 ± 303
MA2.1	347 ± 128

Target cells were pre-incubated with nothing or with Hu-PBL-SCID sera followed by incubation with monoclonal HLA antibody as described in Table 2. The values represented in Table 3 are mean channel fluorescence ± S.E.M. from 3 separate experiments.

HLA-A2 positive PBLs that had been pre-incubated with a saturating concentration of alloantibody-containing Hu-PBL-SCID sera did not block the binding of the HLA-specific monoclonal antibodies (Table 2). None of the monoclonal antibodies blocked the binding of the Hu-PBL-SCID alloantibodies as measured by flow cytometry. The reverse was also true, target cells pre-incubated with HLA-specific monoclonal antibodies were still able to react with Hu-PBL-SCID alloantisera (Table 3).

DISCUSSION

A "humanized" SCID mouse model of human platelet transfusion was used to study the effect of various monoclonal anti-HLA antibodies on

alloimmunization. Whereas the transfusion of untreated platelets induced high levels of alloantibody, platelets presensitized with monoclonal HLA antibodies, but not platelet-specific antibodies, induced virtually no alloantibody in response to five untreated platelet transfusions. The HLA fine specificity of these antibodies and their ability to fix complement did not correlate with the inhibition of the alloimmune response in the model used for the present invention.

Immune modulation by antigen specific polyclonal IgG has been well documented and is thought to occur by means of crosslinking B cell surface Ig with Fc receptors, resulting in the down-regulation of antibody responses. This type of immune regulation requires intact IgG. Antigen-specific polyclonal antibodies have been successfully employed to inhibit a variety of immune responses, including large systems such as red cells as well as viruses and bacterial antigens (Crow AR, et al., *Br J Haematol* **104**:919, 1999). Antigen/antibody complexes have been shown to negatively regulate B cell responses by co-crosslinking surface antigen receptors with Fc receptors. This mode of immune suppression has been used clinically to prevent hemolytic disease of the newborn by administration of anti-D IgG to Rh negative women. The ability of these monoclonal HLA antibodies to negatively regulate B cell antibody production via this negative feedback mechanism is not required for the inhibition seen in the present invention.

Transfusion experiments in rats and Hu-PBL-SCID mice (Crow AR, et al., *Br J Haematol* **104**:919, 1999) have demonstrated that injection of platelets presensitized (IgG coated) with polyclonal sera reactive with the hypervariable (α_1 domain) region of

MHC class 1 could prevent alloimmunization to further transfusions. In the rat models of blood transfusions, the inhibition was linked to cell antigen specific antibodies resulting in FcR-mediated B cell immune suppression or to contamination with an anti-IgG rheumatoid factor-like antibody also resulting in B cell inhibition. In the present invention, it is demonstrated through the use of F(ab')2 fragments of anti-HLA-A,B,C, that the Fc portion of IgG was not required for the alloimmune inhibition. Since the Fc portion of IgG is necessary for co-crosslinking FcR and the BCR, the immune modulation seen here is independent of B cell FcR mediated down-regulation. Also, platelets presensitized with monoclonal antibody to CD32 and CD42a did not result in a decrease in anti-HLA alloantibody production, as would be expected if this immune modulation was due to down-regulation of B cells by FcR crosslinking with the platelet/antibody complexes. Furthermore, the anti-HLA antibodies were equally effective at inhibiting the alloimmune response to further transfusions regardless of their ability to bind host FcR.

Antibody-coated cells are susceptible to complement-mediated lysis or clearance by the reticuloendothelial system. While the W6/32 (HLA-A,B,C) antibody is complement-fixing, the antibodies MA2.1 (HLA-A2) and L368 ($_2$M) are not, and thus complement-dependent platelet clearance is not the mechanism for the immunosuppression observed. Furthermore, the platelet-specific antibodies are complement-fixing as well, and mice challenged with these treated platelets induced a strong anti-HLA alloantibody response to further untreated platelet transfusions. Also, only the first platelet challenge was treated with the monoclonal antibodies, all

subsequent transfusions being with untreated platelets.

The monoclonal HLA-specific antibodies might have blocked the epitopes on Class I to which the patients are immunized, in effect "masking" the HLA Class I on the transfused platelets from the reticuloendothelial system (RES) of the Hu-PBL-SCID mice. The HLA-specific monoclonal antibodies did not down-modulate HLA Class I expression and did not block the binding of anti-HLA alloantisera from Hu-PBL-SCID to target cells. Nor did alloantisera block the binding of the monoclonal antibodies to platelets. Nonetheless, presensitization of platelets with HLA antibodies may allow the platelets to go undetected by the host immune system and thus prevent an immune response. This may explain why challenge with untreated platelets after the first exposure to pretreated platelets did not elicit an antibody response.

Although leucodepletion may prevent primary alloimmune responses to platelet transfusions, it is not completely effective and may not be able to prevent further alloimmunization. Antibody-mediated inhibition of the human alloimmune response may provide a useful regime for inhibiting the incidence of alloimmunization to platelet transfusions.

The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

Murine monoclonal antibodies

The hybridomas W6/32 (IgG_{2a}, anti-HLA-A,B,C), MA2.1 (IgG_1, anti-HLA-A2/B17), L368 (IgG_1k, anti-$_2$

microglobulin), and IV.3 ($IgG_{2b}k$, anti-FcγRII) were obtained from A.T.C.C. (Manassas, VA). Antibodies were used as tissue culture supernatants. The monoclonal anti-CD42a (AN51, IgG_{2a} k) was obtained from Dako Diagnostics (Mississauga, ON). The control murine IgG was purchased from Cedarlane (Hornby, ON).

The F(ab')2 fragment of W6/32 was prepared by incubating 2 mg/ml purified antibody in 0.2M acetate buffer (pH 4.5) with 0.1 mg/ml pepsin for 20 h at 37°C. Digestion was stopped by the addition of 2M Tris base. Free Fc fragments and whole IgG were removed by passage of the digested product through a Protein-A Sepharose™ column. F(ab')2 purity was found to be >99% by gel scanning and it was again passed over Protein-A Sepharose™ to remove the remaining 1% contaminants. The ability of the F(ab')2 fragments to bind platelet HLA was confirmed by flow cytometry (Fig. 1F).

EXAMPLE 2
SCID mice

C.B.17 SCID female virgin mice (6-10 weeks of age) were obtained from Charles River Laboratories (Montreal, PQ) and were housed under gnotobiotic conditions in the St. Michael's Hospital research vivarium. Blood from the tail vein (300 µl) was collected into untreated microvette tubes (Sarstedt, Montreal, PQ). Serum was separated after incubation at 22°C for 2 h. Serum levels of endogenous murine IgG were determined by ELISA and mice with a serum level of greater than 10 µg/ml were excluded from the study.

EXAMPLE 3
Reconstitution of SCID mice

PBL were obtained by Percoll separation of whole blood from female blood donors with stable, low levels of circulating HLA Class I alloantibodies due to prior pregnancy. The first donor was blood group O, HLA-A1, A3, B7 and B37 positive and had circulating anti-HLA-A2 and -B5 alloantibodies. The second donor was blood group A, and circulating levels of broad polyspecific alloantibodies. All SCID mice were injected with 20 μl of anti-asialo GM_1 antiserum (Wako Pure Chemical Industries Ltd., Dallas, TX) 1 day prior to reconstitution and were exposed to 200 cGy of γ-irradiation just prior to reconstitution to enhance cellular engraftment as previously described. Human PBL ($1x10^7$/mouse) were isolated and injected into the peritoneal cavity as previously described (Crow AR, et al., *Br J Haematol* **104**:919, 1999).

EXAMPLE 4
Reconstituted SCID mouse challenge

Challenge platelets were obtained from buffy coats in CP2D bags and isolated by centrifugation of the platelet-rich plasma at 200 x g. Hu-PBL-SCID mice were challenged with γ-irradiated (2,500 cGy) human platelets from five random HLA-A2 positive donors with or pooled platelets from five random donors with different Class I alleles. The first challenge consisted of $4x10^8$ platelets/mouse (equivalent to 2 transfusions in a human). Subsequent challenges were with $2x10^8$ untreated platelets (equivalent to 1 human transfusion), twice weekly for three weeks. In specified groups of mice, the platelets used for the first challenge consisted of platelets presensitized with saturating levels of monoclonal antibody (as assessed by flow cytometry; Figs. 1A to 1G), or 1

μg/ml control murine IgG, for 0.5 h at 22°C. Platelets were then washed twice with phosphate buffered saline (PBS; pH 7.2) and resuspended in PBS. Mice that received antibody-sensitized platelets only did so on the day of engraftment; the five subsequent transfusions were with untreated platelets.

EXAMPLE 5
Antibody detection

Mouse and human serum IgG levels were assessed by ELISA. Alloantibodies were detected by flow cytometry as previously described (Crow AR, et al., *Br J Haematol* **104**:919, 1999). Briefly, sera from Hu-PBL-SCID mice were diluted **1**:10 in PBS and incubated with $2x10^5$ pooled lymphocytes (obtained from the same source as the platelet challenges). The cells were then washed twice and incubated with 1 μg/ml of affinity-purified fluorescein isothiocyanate (FITC)-conjugated F(ab')2 anti-human IgG Fcγ-specific antibody (Tago Biosource, Camarillo, CA). The cells were then washed twice and fixed in 1% paraformaldehyde in PBS. For monoclonal antibody saturation assessment, platelets were incubated in 100 μl PBS with various serial dilutions of antibody for 0.5 h, washed and incubated with 1 μg/ml FITC-conjugated F(ab')2 anti-mouse IgG (Cedarlane, Hornby, ON). Ten thousand events were acquired and analyzed by a FACSort™ flow cytometer (Becton-Dickinson, San Jose, CA) operating at 15 mW power. Background staining was assessed by comparison with serum obtained from each animal prior to any manipulation.

For the steric hindrance studies, HLA-A2 positive PBLs were incubated with saturating levels of 1) alloantibody-containing Hu-PBL-SCID sera or 2) monoclonal HLA antibody for 1h at room temp. After

washing twice with PBS, the cells were then incubated with 1) monoclonal HLA antibody or 2) Hu-PBL-SCID sera respectively. Following washing, cells from 1) were incubated with goat F(ab')2 anti-mouse IgG-FITC, and cells from 2) were incubated with goat F(ab')2 anti-human IgG-FITC. Cells were then analyzed by flow cytometry.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

What is claimed is:

1. A method for preventing HLA alloimmune response to platelet transfusion, said method comprising the step of presensitizing platelets with at least one monoclonal antibody against HLA, a portion thereof or β2-microglobulin, wherein said platelets if administered to a patient prevent an HLA alloimmune response from said patient.

2. The method of claim 1, wherein said at least one monoclonal antibody is W6/32, L368, and MA2.1.

3. A method for inhibiting an HLA alloimmune response to platelet transfusion, said method comprising the steps of:

a) presensitizing platelets with at least one monoclonal antibody against HLA, a portion thereof or β2-microglobulin;

b) transfusing with the presensitized platelets of step a) to a patient, said presensitized platelets inhibiting an HLA alloimmune response from said patient.

4. The method of claim 3, wherein said HLA alloimmune response is still inhibited after at least two transfusions from said patient.

5. A method for preventing refractoriness to subsequent transfusions in an alloimmunized patient, comprising the steps of:

a) presensitizing platelets with at least one monoclonal antibody against HLA, a portion thereof or β2-microglobulin; and

b) transfusing the alloimmunized patient with the presensitized platelets of step a), the presensitized platelets preventing refractoriness to the transfusion.

ABSTRACT OF THE INVENTION

The present invention relates to a method for preventing HLA alloimmune response to platelet transfusion. The method comprises the step of presensitizing platelets with at least one monoclonal HLA antibody. The platelets if administered to a patient prevent an HLA alloimmune response from said patient.

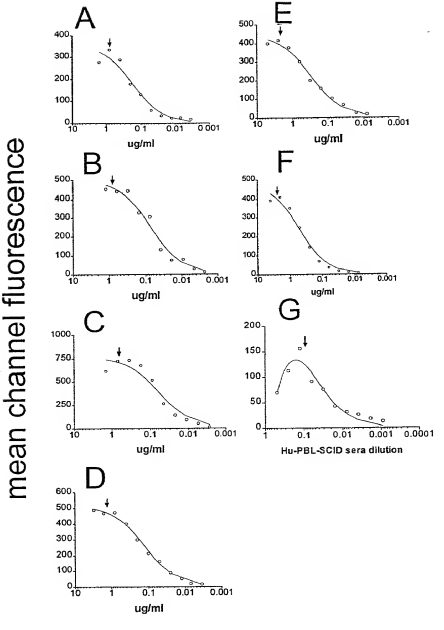
mean channel fluorescence
A
B
C
D
E
F
G
ug/ml
Hu-PBL-SCID sera dilution

Fig. 1

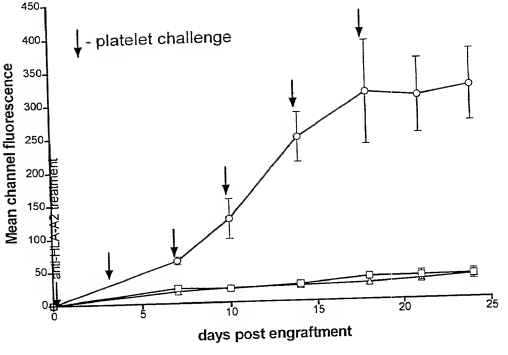
- platelet challenge
Mean channel fluorescence
anti-HLA-A2 treatment
days post engraftment
0
50
100
150
200
250
300
350
400
450
5
10
15
20
25

Fig. 2

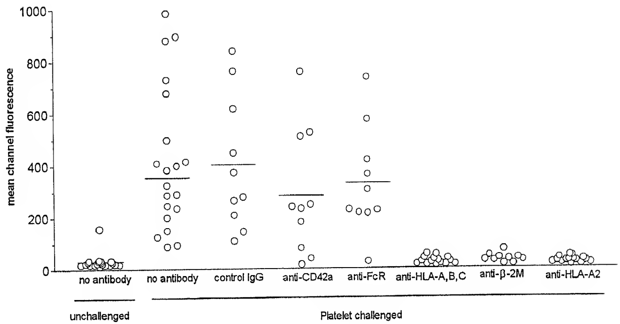
mean channel fluorescence
1000
800
600
400
200
0
no antibody
no antibody
control IgG
anti-CD42a
anti-FcR
anti-HLA-A,B,C
anti-β-2M
anti-HLA-A2
unchallenged
Platelet challenged

Fig. 3

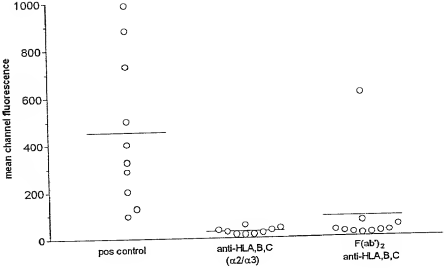
1000
800
600
400
200
0
mean channel fluorescence
pos control
anti-HLA,B,C
(α2/α3)
F(ab)2
anti-HLA,B,C

Fig. 4